PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68 | A2

(11) International Publication Number: **WO 00/29619**

(43) International Publication Date: 25 May 2000 (25.05.00)

(21) International Application Number: PCT/US99/26673

(22) International Filing Date: 12 November 1999 (12.11.99)

(30) Priority Data:
60/108,415 13 November 1998 (13.11.98) US

(71) Applicant *(for all designated States except US)*: MOSAIC TECHNOLOGIES [US/US]; 1106 Commonwealth Avenue, Boston, MA 02215 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BOLES, T., Christian [US/US]; 5 Judith Lane, No. 6, Waltham, MA 02454 (US). MUIR, Andrew, R. [US/US]; 481 Jerusalem Road, Cohasset, MA 02025 (US).

(74) Agents: HOGLE, Doreen, M. et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MULTIELEMENT ANALYTICAL DEVICE FOR ASSAY OF NUCLEIC ACID SEQUENCES AND USES THEREFORE

(57) Abstract

A multielement analytical device is described which can be used in highly efficient multiplexed assay for detecting the presence of a target nucleic acid sequence in a plurality of test samples. The device contains a plurality of elements, each containing at least one essentially flat surface in which an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured so that the essentially flat surface of each element can be simultaneously immersed in a single test sample container.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

MULTIELEMENT ANALYTICAL DEVICE FOR ASSAY OF NUCLEIC ACID SEQUENCES AND USES THEREFORE

RELATED APPLICATION(S)

This application claims the benefit of United States Provisional Application entitled "Multi Element Analytical Device and Methods of Use", filed on November 13, 1998 and assigned Serial Number 60/108,415. The teachings contained within that application, including all text, figures and references cited therein, are herey incorporated by reference.

BACKGROUND OF THE INVENTION

The principle of hybridization is the property upon which most practical methods of synthesizing and detecting nucleic acid sequences are based. Nucleic acid hybridization assays rely on the ability of nucleic acid strands to pair with their complementary sequences under suitable conditions. In general, hybridization assays involve incubating a nucleic acid sequence complementary to a nucleic acid sequence of interest with a sample thought to contain the sequence of interest, and identifying the presence of the sequence of interest by detecting the complex formed by the hybridization of the two sequences. Such assays have been widely used to identify, purify and detect nucleic acids for a variety of purposes (see Wetmur, 1991, *Critical Reviews in Biochemistry and Molecular Biology* 26, 227-259).

The Polymerase Chain Reaction (PCR) is a process which amplifies the quantity of a nucleic acid sequence. PCR utilizes a pair of nucleic acid primers, each of which is complementary to an end of the nucleic acid sequence being amplified. As the primers hybridize to the nucleic acid being amplified, the nucleic acid is extended, and a sequence complementary to the sequence to be amplified is synthesized. Each time this cycle is repeated, the amount of the sequence being amplified doubles in quantity. Thus, PCR provides the capability of increasing even a minute quantity of a specific nucleic acid to a level at which it can be detected.

a minute quantity of a specific nucleic acid to a level at which it can be detected. Hybridization assays utilizing PCR amplification prior to a nucleic acid detection step are extremely versatile. Such assays are used to detect microbial and viral pathogens, to diagnose cancer and genetic diseases, as well as to identify individuals susceptible to particular conditions due to their genetic make-up (see Saiki, R.K. *et al.*, *Science 239*:487 (1988)).

Furthermore, as described in United States Patent No. 5,641,658 entitled "Method for Performing Amplification of Nucleic Acid With Two Primers Bound to a Single Solid Support", the rapid and reproducible detection of a sequence which has been hybridized and/or amplified is facilitated by performing those processes on a solid support. The disclosed technique permits multiple analytes in a test sample to be amplified on a surface, and then individually detected at localized sites. Nucleic acid sequences localized, for example, on the end surface of individual optical glass fibers have been used to detect minute quantities of a nucleic acid sequence in a variety of test samples.

However, certain inherent requirements of multiple-fiber optical bundles make the extension of a nucleic acid assay performed with a single glass fiber to a multiplexed nucleic acid assay utilizing multiple-fiber optical bundles both difficult and potentially quite expensive. For example, individually localizing primers at individual fiber amplification sites in a large assembled fiber bundle would be impractical, as would pre-coating large numbers of primers on the individual fibers and then assembling them into fiber bundles. Thus, there exists a need for improved devices and methods for use in multiplexed amplification reactions performed on solid supports for clinical diagnostic or research applications.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the discovery of a multielement analytical device useful in a multiplex assay for detecting the presence of a target nucleic acid sequence in test samples. This device is both simple and

inexpensive, and can be readily manufactured, even as a single-component disposable unit.

In one aspect, the present invention pertains to an analytical device with a plurality of elements, each element containing at least one essentially flat surface with an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured so that the essentially flat surface of each element can be simultaneously immersed in a single test sample container.

In a preferred embodiment, the elements are cylindrical. In a preferred embodiment, the elements are solid. In a particularly preferred embodiment, the elements are hollow.

In preferred embodiments, the device contains 96, 192, 288, or 384 elements.

In a preferred embodiment, the test sample container is a 96-well microtiter plate.

In preferred embodiments, an array contains 6, 12, 24, 36, 48, 60, 72, 84, 96 or 108 amplification sites.

In a preferred embodiment, the device is formed from a polymer.

In an additional embodiment, the device also contains a connecting element.

In a preferred embodiment, the essentially flat surface of the element has a surface design which is a rough surface, a pitted surface, a smooth surface surrounded by an annular ring, a smooth surface with discrete sections formed by raised ridges, a smooth surface with discrete sections formed by indented channels or any combination of those surface designs.

In a preferred embodiment, the device also contains an acrylamide gel coating at the amplification sites.

In a preferred embodiment, the surface of the amplification site is positively charged.

In another aspect, the present invention pertains to a multiplexed assay method for detecting the presence of a target nucleic acid sequence in test samples in which an analytical device with a plurality of elements, each element containing at least one essentially flat surface with an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured such that the at

least one flat surface of each element can be simultaneously immersed in a single test sample container, is introduced into a single test sample container such that the amplification sites are simultaneously in contact with the test samples under conditions suitable for hybridization to occur, contact between the amplification sites and the test samples is maintained for sufficient time for hybridization to occur, the hybridized target nucleic acid sequences are amplified, and the presence of the amplified target nucleic acid sequences is detected.

In a preferred embodiment, the analytical device is separated from the test container prior to the amplification step.

In a preferred embodiment, the amplified target nucleic acid sequences are optically detected. In a particularly preferred embodiment, the amplified target nucleic acid sequences are detected by fluorescence.

In a preferred embodiment, the target nucleic acid sequence is amplified about 10^4 to 10^6 fold.

In another aspect, the invention pertains to a method of making an analytical device with a plurality of elements, each element containing at least one essentially flat surface with an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured such that the at least one flat surface of each element can be simultaneously immersed in a single test sample container by molding, machining from bulk material, assembling from smaller parts, and any combinations thereof. In a particularly preferred embodiment, the analytical device is made by injection molding.

In yet another aspect, the invention pertains to a kit containing an analytical device with a plurality of elements, each element containing at least one essentially flat surface with an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured such that the at least one flat surface of each element can be simultaneously immersed in a single test sample container. In a preferred embodiment, the kit also contains a test sample container.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

FIGS. 1A and 1B depict an illustration of an embodiment of the multielement analytical device with a corresponding test sample container.

FIGS. 1C and 1D depict illustrations of two embodiments of an individual element shown with the corresponding portion of a test sample container in enlarged section.

FIG. 2 depicts an array of amplification sites contained on the essentially flat surface of an element of the multielement analytical device.

FIGS. 3A, 3B, 3C and 3D depict alternative surface finishes for the essentially flat surface of the element of the analytical device.

FIGS. 4A, 4B and 4C depict three embodiments of an individual element of the multielement analytical device shown with the corresponding portion of a test sample container and three styles of connecting components.

FIGS. 5A, 5B and 5C illustrate a first cycle in a surface bound amplification reaction.

FIGS. 6A, 6B and 6C illustrate a second cycle in a surface bound amplification reaction.

FIGS. 7A,7B and 7C illustrate a third cycle in a surface bound amplification reaction.

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein pertains to apparatus, methods and kits useful in the hybridization and amplification analysis of nucleic acids. Specifically, a multielement analytical device is described which can be used in a highly efficient multiplexed assay for detecting the presence of a target nucleic acid sequence in test samples. The device contains a plurality of elements, each element containing at least one essentially flat surface with an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured so that the essentially flat surface of each element can be simultaneously immersed in a single test sample container. The methods of the invention describe the use of the device in nucleic acid hybridization assays, particularly those utilizing a nucleic acid amplification process.

The analytical device of the invention contains a plurality of elements. The language "element" is intended to include that portion of the analytical device configured for insertion into a test sample container. An element can be of any form and of any dimension. In a preferred embodiment, the form and dimension of the element correspond to the form and dimension of a complementary test sample container. An element can be formed as a single unit with the other portions of the analytical device, or alternatively, it can be formed as a discrete unit and subsequently joined to the analytical device. An analytical device of the invention can contain any number of elements greater than one. Preferred embodiments of the invention contain 96, 192, 288, or 384 elements.

In a particularly preferred embodiment as depicted in FIG. 1A, the analytical device contains ninety-six (96) essentially cylindrical elements configured so that each can be inserted into the corresponding ninety-six (96) wells of a standard microtiter plate. Corning Costar (Acton, Ma.). In this embodiment, the elements can be described as "post shaped" as can best be seen in FIG. 1C, with each separate post considered to be a separate element. The elements may be either solid or hollow.

Each element contains at least one essentially flat surface. The essentially flat surfaces of the elements of an analytical device are placed so that all the essentially flat surfaces contained on a single device can be simultaneously

immersed in a single test sample container. The language "essentially flat surface" is intended to include any surface which is relatively level. It is not required that the essentially flat surface of each element be perfectly contained within a single plane, rather, it is merely required that all the essentially flat surfaces of the elements of a single device be sufficiently uniform that they can be simultaneously immersed in a single test sample container. Thus, it will be understood that a significant amount of deviation from perfectly level in the essentially flat surface can be tolerated.

In the 96-element preferred embodiment described previously, the essentially flat surface is located at one end of the post, on a plane which is perpendicular to the longest portion of the post. The opposite end of the post is attached to the rectangular surface of the analytical device. When the analytical device is properly positioned into the microtiter plate, each post enters an individual well of the microtiter plate. If a well of the microtiter plate contains a sufficient amount of a test sample, the flat surface of the post will be immersed in the test sample. Furthermore, in this embodiment and others, the diameter of the portion of each post containing the essentially flat surface, can be so closely matched to the internal diameter of a standard microtiter plate that when the multielement device is inserted into the plate, the rate of evaporation of the test sample will be reduced.

The apparatus, methods and kits described in this application utilize a solid support or an amplification surface for the amplification and detection of a target nucleic acid sequence as can be seen in FIG. 2. Thus, within this application, the language "solid support" or "amplification surface" are used essentially interchangeably and are intended to include the essentially flat surface of the element when it has been provided with alignment features to facilitate the precise positioning of nucleic acid sequences. Preferably, the supports or surfaces useful in the present invention comprise inert or inactive materials that do not react with components of the amplification reaction, or interfere with the amplification reaction. Such materials include, for example, epoxy silane, polystyrene, polycarbonate, polypropylene or other plastics, derivatized silica, nylon or latex. Alternatively, a support material can be treated, or coated, with a inert material. This type of surface allows for the delineation of areas of the surface directed to two or

more distinct nucleic acid sequences, e.g., a pair composed of a first sequence and a second sequence.

The amplification surface of the present invention can be of any size or shape, and can contain any number of amplification sites arranged in any manner. The language "amplification site" is intended to include a location on an amplification surface which contains a covalently bonded pair of oligonucleotide primers. In preferred embodiments of the invention, the amplification surface contains 6, 12, 24, 36, 48, 60, 72, 84, 96 or 108 amplification sites. In the 96-element preferred embodiment described previously, the amplification surface can contain any number of amplification sites, but preferably contains 24 amplification sites. In a preferred embodiment, the amplification sites are arranged in a grid type pattern of amplification pixels suitable for amplifying multiple nucleic acid sequences.

Each amplification site is provided with a pair of oligonucleotide primers which are covalently attached to the amplification surface. The language "primer" is intended to include an oligonucleotide of any sequence or length obtained from any source. In a preferred embodiment, these primers are relatively short in length and of single stranded nucleic acid, most preferably deoxyribonucleic acid (hereinafter "DNA"). In a particularly preferred embodiment, the primers are 15 to 50 base pairs in length. The sequence of each oligonucleotide primer is complementary to the sequence at one end of the target nucleic acid. For example, when the target nucleic acid is DNA, one oligonucleotide primer is complementary to one strand of the target DNA, whereas the other oligonucleotide primer is complementary to the opposite strand. The 5' ends of both primers are attached to the amplification surface, while the 3' ends of both primers are left unattached, so that they are free to participate in the PCR reaction, e.g. to hybridize to target nucleic acids. The surface density of primers is sufficient for the amplified product from the reaction to span between primer supports in the form of a double stranded nucleic acid bridge.

The nature of the amplification surface can affect the amplification process. As described previously, the amplification surface is preferably formed of inert materials. But in addition, the surface can be coated with a material that modifies its

properties in advantageous ways, either for attaching primers, or for permitting the amplification reaction to proceed without chemical modification or interference. Thus, the amplification surface of the analytical device may be treated with one or more secondary substances which, for example, render the surface hydrophilic or hydrophobic, aid in the adhesion of one or more other substances, reduce the adhesion of one or more other substances, or promote or prevent one or more chemical reactions.

The amplification surface may be coated with a large variety of polymers including many widely available monomers and crosslinkers. In a preferred embodiment, an acrylamide polymer gel is applied. Such a method for treating the amplification surface includes copolymerizing an acrylamide-modified oligonuceotide into a polyacrylamide copolymer such as Acrydite™ phosphoramidite (Mosaic Technologies, Boston, MA) with amino acrylamide on the surface. Alternatively, the surface can be modified by polymerization with low levels of cystaminebisacrylamide, followed by reduction with tris(2-carboxyethyl) phosphine (TCEP). This treatment permits the introduction of a controlled level of hydrogen sulfide (-SH) groups onto the surface and allows for further modifications, for example, the incorporation of very hydrophobic monomers in nonaqueous solution.

In addition, amplification surfaces with a net positive electrical charge are preferred. A positive charge of moderate strength is particularly preferred, so that non-specific binding of nucleic acids is avoided. Optimization of the surface electrical charge can also be achieved using the materials and coatings described above.

When possible, it is generally advantageous to increase the total surface area of an amplification surface. This can be accomplished by applying acrylamide polymer with large pores, or acrylamide polymer which has been copolymerized with inert submicron silica beads. Silica beads can be dispersed from such a polymer matrix using a mild sonication process.

A review of the figures, particularly FIGS. 1, 2, 3 and 4 will facilitate an understanding of the analytical device of the present invention. FIG. 1A is an

illustration of an embodiment of the multielement analytical device, while FIG. 1B is an illustration of a corresponding test sample container. In the embodiment depicted in FIG. 1A, the device is a unitary single component disposable device with a form and dimensions complementary to the form and dimensions of the corresponding test sample container, intended to represent a standard microtiter plate. The multielement device features a rectangular surface 1 attached to a number of elements 2. These elements are configured for ready insertion into the wells of the microtiter plate 3. In this embodiment, each cylindrical post of the multielement device constitutes an element of the device. Each individual element contains an essentially flat surface 4 which, when the device is properly positioned into the microtiter plate, will enter an individual well of the microtiter plate. If the well of the microtiter plate contains a sufficient amount of a test sample, the flat surface of the post will be immersed in the test sample. Moreover, all the flat surfaces of the elements of the multielement device can be simultaneously placed in the wells of the microtiter plate. If all the wells of the microtiter plate contain sufficient amounts of test samples, the flat surfaces of all the posts of the multielement device will be simultaneously immersed in the test samples.

FIGS. 1C and 1D each depict an individual element 2 of an analytical device of the invention which has been inserted into the well 3 of a test sample container. The element of FIG. 1C is of solid construction, while the element of FIG.1D is of hollow construction.

FIG. 2 depicts an array of amplification sites 5 located on the essentially flat surface 4 of an element 2 of the multielement analytical device of the invention.

FIGS. 3A-3D illustrate various alternative surface finishes suitable for addition to the essentially flat surface of an element of the invention. As illustrated in FIG. 3A, the surface 4 of an element can be slightly uneven or even quite rough. Such treatment can facilitate the adhesion of surface-bound chemical reagents. As illustrated in FIG. 3B, the entire surface 4 of an element can be enclosed by an annular ring or an equivalent boundary enclosure. Such additions can aid in the retention of fluids upon the surface. As illustrated in FIG. 3C, the surface 4 can be treated to contain small wells or pits. These adaptations can contain reagents and/or

facilitate the adhesion of reagents to the surface of the element. Or, as illustrated in FIG. 3D, the surface 4 can be formed to contain a pattern of raised ridges or a pattern of indented channels. Such additions can segregate different reagents upon the surface of the element. Various combinations of the surface treatments can also be utilized. For example, the annular ring of FIG. 3B can be combined with the segregating patterns contained in FIG. 3D. One skilled in the art would be able to envision many other surface treatments which would facilitate the use of particular methods with the analytical device of the invention. All such treatments are considered to be within the scope of the present invention.

FIGS. 4A, 4B and 4C each depict an individual element 2 of an analytical device of the invention which has been inserted into the well 3 of a test sample container. The elements of FIGS. 4A and 4C are of solid construction, while the element of FIG.4B is of hollow construction. Each figure further depicts a connecting component 6 which can be used to reversibly attach the multielement analytical device to the test sample container. In FIG. 4A, the connecting component is a liquid seal located on the analytical device. In FIG. 4B, the connecting component is a liquid seal placed on the test sample container. In FIG. 4C, the connecting component is a sealing gasket interspersed between the device and the container. One skilled in the art would be able to envision many other connecting components which would allow the analytical device of the invention to be reversibly attached to a test sample container. All such components are considered to be within the scope of the present invention.

The multielement analytical device described can be used in a variety of assays. It is particularly suited for use in a multiplexed assay method for detecting the presence of a target nucleic acid sequence in a plurality of test samples. In such an assay, the elements of a device of the invention are introduced into a test sample container such that the amplification sites are simultaneously in contact with the test samples under conditions suitable for hybridization to occur, contact between the amplification sites and the test samples is maintained for sufficient time for hybridization to occur, the hybridized target nucleic acid sequences are amplified, and the presence of the amplified target nucleic acid sequences is detected.

The language "multiplexed assay" is intended to include the detection of a target nucleic acid sequence in a plurality of test samples by multiple detection devices. The language is also intended to include the detection of multiple target nucleic acid sequences in a plurality of test samples by multiple detection devices. Such detection can occur simultaneously, sequentially or with time overlap. In preferred embodiments, the detection of the multiple sequences occurs simultaneously.

The language "test sample" is intended to include any sample containing a target nucleic acid sequence capable of being amplified. For example, the target nucleic acid sequence can be of mammalian, specifically human, origin such as a gene, gene fragment or gene product. The target nucleic acid sequence can also be of bacterial, viral, parasitic or yeast origin. The test sample can comprise any sample that contains nucleic acid sequences, for example, biological fluids such as blood, urine, cerebral spinal fluid, semen, saliva, stool or perspiration. The test sample can also comprise whole or lysed cells, or tissue such as biopsy material. Also encompassed by this invention are test samples which are to be tested for the presence of nucleic acid sequences as contaminates, such as nucleic acid sequences resulting from bacterial contamination in, for example, chemical extracts and distillates and other suspensions or colloids.

The language "nucleic acid" or "nucleic acid sequence" and their respective plurals are used essentially interchangeably herein and are intended to include "DNA" and ribonucleic acid (hereinafter "RNA"). Both single stranded and double stranded nucleic acids are embraced by this invention. Higher ordered structures of nucleic acids, for example, RNA that has folded upon its linear strand forming a secondary loop structure, are also within the scope of the present invention. Nucleic acid sequences encompassed by the present invention can be from about 3 to about 10,000 nucleotides in length. There is no absolute minimum or maximum length requirement for target nucleic acid sequences, however, the usual range is from about 10 to about 2,000, preferably about 30 to about 1,000, most preferably about 30 to about 100. Similarly, there is no absolute minimum or maximum length requirement for probe nucleic acid sequences, however, a preferred range is from

about 15 to 50, most preferably about 20 to 45. It should also be understood that the nucleic acid sequences of the present invention may be embedded within longer strands of nucleic acids or associated with other molecules. The directionality of the nucleic acids of the current invention may be either 5' to 3' or the reverse, that is, 3' to 5'.

The language "modified nucleic acid" is intended to include a DNA or RNA nucleic acid molecule that contains chemically modified nucleotides. The language "nucleic acid analogue" is intended to include non-nucleic acid molecules that can engage in base-pairing interactions with conventional nucleic acids. These modified bases and nucleic acid analogues are considered to be within the scope of the instant invention. For example, nucleotides containing deazaguaine and uracil bases can be used in place of guanine and thymine, respectively, to decrease the thermal stability of hybridized probes. Similarly, 5-methylcytosine can be substituted for cytosine in hybrids if increased thermal stability is desired. Modification to the sugar moiety can also occur and is embraced by the present invention. For example, modification to the ribose sugar moiety through the addition of 2'-O-methyl groups can be used to reduce the nuclease susceptibility of RNA molecules. Modifications occurring with different moieties of the nucleic acid backbone are also within the scope of this invention. For example, the use of methyl phosphate, methyl phosphonate or phosphorothioate linkages to remove negative charges from the phosphodiester backbone can be used.

The best known example of a nucleic acid analogue is peptide nucleic acid (PNA), in which standard DNA bases are attached to a modified peptide backbone comprised of repeating N-(2-aminoethyl)glycine units (Nielsen *et al.*, *Science* vol. 254, pp. 1497-1500, 1991). The peptide backbone is capable of holding the bases at the proper distance to base pair with standard DNA and RNA single strands. PNA-DNA hybrid duplexes are much stronger than equivalent DNA-DNA duplexes, probably due to the fact that there are no negatively charged phosphodiester linkages in the PNA strand. In addition, because of their unusual structure PNAs are very resistant to nuclease degradation. It will be apparent to those skilled in the art that

similar design strategies can be used to construct other nucleic acid analogues that will have useful properties for nucleic acid hybridization and amplification assays.

The language "base-pairing" is intended to include those reactions which occur in an antiparallel manner as well as those which occur in a parallel fashion. Base-pairing itself is understood to essentially follow a complementary pattern wherein a purine pairs with a pyrimidine via hydrogen bonds. More particularly, it is understood that complementary base-pairing of individual base pairs generally follows Chargaff's Rule wherein an adenine pairs with a thymine (or uracil) and guanine pairs with cytosine. However, there are modified bases which account for unconventional base-pairing and these are considered to be within the scope of the instant invention.

The language "hybridize" or "hybridization" is intended to include admixing of at least two nucleic acid sequences under conditions such that when at least two complementary nucleic acid sequences are present, they will form a double stranded structure through base-pairing. The language "complement" or "complementary" is intended to describe nucleic acid sequences which can form a double stranded structure through base-pairing. Mismatches are permitted in the instant invention. Nucleotide mismatch can affect the affinity between nucleic acid sequences. The greater the mismatch between nucleic acid sequences, generally the lower the affinity between them as compared to perfectly matched nucleic acid sequences. Generally, the greater the mismatch between nucleic acid sequences, the more readily hybridization that exists between them can be disrupted.
for obtaining affinity ligands against virtually any target molecule.
for purification and detection of targets that bind to or are bound by them.

Altering the temperature at which the hybridization reaction occurs is a common method for modulating hybridization complex binding affinity. For example, at temperatures above the melting temperature (Tm) of the hybridization complex, binding affinity will be low. Similarly, at temperatures below the Tm, binding affinity will be substantially higher. Therefore, if the Tm of a hybridization complex containing mismatches is determined and compared with the Tms of complexes with shorter lengths of perfectly matched nucleotides, the effective

pairing length of complexes with mismatches can be ascertained and applied to the present invention. Thus, the methods of the present invention can be optimized for particular target sequences both by selection of probe sequences and by selection of assay temperature parameters.

The language "amplification" is intended to include any reaction in which the total quantity of a target nucleic acid sequence is increased. In a preferred embodiment, the PCR process is used to increase the quantity of a target nucleic acid to a detectable level.

Each step in the methods of the present invention can be performed manually, or alternatively, in an automated instrument, or in a series of automated instruments. Moreover, the various steps of the methods can be performed independently of one another, while utilizing different reagents and instruments. United States Patent No. 5,641,658 to Adams *et al.*, and the United States applications with Serial Numbers 08/800,840 filed February 14, 1997 and 09/327,083 filed June 4, 1999, which are hereby incorporated by reference in their entireties, describe methods and apparatus for performing nucleic acid hybridization and amplification processes on a solid support.

According to one embodiment of the present invention, a target nucleic acid sequence in a test sample is amplified, detected, and can be quantified, using pairs of primers attached to a surface contacting the sample and, optionally, other chemical reagents. Each pair of primers is homologous to a complementary end of the length of a target sequence. When amplification conditions are imposed, an amplified target nucleic acid sequence is formed, and attached to the surface by extension from the primers so attached. Because the primer pair is selected to be specific to the target sequence contained in the test sample, surface bound amplificate forms only if the target sequence is present in the test sample. Amplification can be performed using any commercially available instrument, for example, the thermocyclers produced by MJ Research (Waltham, MA) and Perkin Elmer Corporation (Norwalk, CN) are well suited for use with the apparatus and methods of the invention.

The amplificate so formed can be detected conveniently by optical means, particularly, if the amplificate is labeled. The optical detection scheme can operate

directly on the reagent-binding surface, or from underneath the surface, by transmission through the post material. Labeling techniques include using labeled nucleic acids in the PCR mixture, including a probe which is specific to the amplificate of the target sequence in the mixture and which is detectable after being hybridized to the amplificate, or adding such a detectable probe after the amplification phase of the analysis is complete. A variety of labeled probes for such purposes is well known within the art. The optical signal, if present, can be detected by a variety of known optical detection techniques, such as photodiodes, photomultipliers, television cameras and CCD arrays.

When the detection scheme is one based on fluorescence of a fluorogenic substance, such fluorescence can be induced by irradiating the surface bound amplificate with excitation radiation, such as that from an incandescent lamp, a discharge lamp, a laser, or utilizing other irradiation methods known within the art. The GMS 418 Optical Imaging Microarray Reader (Genetics Microsystems, Woburn, MA) is one example of a device well suited to provide high sensitivity fluorescence detection for the methods of the invention.

The amplification process can be localized to a given area by attaching the primer pair only at a given area, such that the surface bound amplificate only forms there, and the resultant optical signal is localized and can be detected at the predetermined location.

Moreover, multiple target sequences within the test sample can be tested for by independently attaching multiple primer pairs in different areas of the amplification surface. Each primer pair is homologous to a given sequence within a single length of target nucleic acid, or to sequences in two or more lengths of target nucleic acids in the test sample. After amplification conditions have been imposed, and the simultaneous or subsequent hybridization of an optically detectable nucleic acid is effected, the presence or absence of multiple different target sequences can be determined from the presence or absence of optical signals from the appropriate localized areas of the surface. It is particularly advantageous to position the localized primer pairs on the surface such that the optically detectable labeled amplificate is formed into a group that is easily detected by optical schemes able to

detect spatially distinct optical signals. Such multiple optical signals in parallel can be detected by imaging the optical pattern onto an area sensitive optical detector such as a television camera, or CCD array, or using other methods known within the art. Alternatively, the pattern of multiple optically detectable signals may be detected sequentially, such as by individually imaging each signal in turn onto a detector. Detectors such as photodiodes, photomultipliers and other known detection systems can be used. Moreover, such detection may be achieved by masking the optical signals with one or more spatial filters, and sequentially permitting each individually to be detected by the optical detector.

Other optical methods of detection including phosphorescence, luminescence, chemiluminescence, absorption, surface plasmon resonance, reflectance change, rotation of optical polarization, and other known techniques are also useful and encompassed within the scope of this invention.

Furthermore, multiplexed assays are particularly included within the methods of this invention. In such assays, the target nucleic acid sequences in a plurality of test samples are detected by multiple detection devices. Similarly, multiple target nucleic acid sequences can be detected in a plurality of test samples by multiple detection devices. These detections can occur either simultaneously, sequentially or with time overlap. All devices and methods previously described are also suitable for use in multiplexed assays.

The surface to which the primer pairs are bound may be heated and/or cooled to effect thermocycling as part of the amplification conditions. A passive device can be mounted against a heating/cooling fixture such that the combination may be completely or partially immersed in liquid and the device driven through temperature cycles suitable for PCR amplification. The fluid may be a bulk solution or several solutions, each bathing one or more of the reaction surfaces on the device. Heating may be effected by, for example, applying heated material to the surface supporting the primers, applying electrical joule heating, applying electrical peltier heating, applying electromagnetic radiation, and other techniques known to those of skill in the relevant art. Cooling may also be effected by, for example, applying cooled material, applying electrical peltier cooling, permitting the adiabatic expansion or

evaporation of a liquid, conducting heat away from the surface into the test sample, and other techniques known to those of skill in the relevant art. Several companies including Stratagine, San Diego, CA. and M. J. Research, Waltham, MA. Produce commercial products useful for these embodiments

Additional components may be included for detecting the temperature of the amplification surface, and/or of the test sample within the vicinity of the surface, for example, thermocouples, thermistors, resistance thermometers, semiconducting devices, temperature sensitive optical elements, temperature sensitive magnetic materials, thermal expansion devices, and other known temperature sensing devices. In some embodiments, the multielement device contains heating and cooling components so that PCR can be performed without the use of a thermocycler. Such temperature sensing components may be combined with heating and cooling components to effect temperature control in ways well known to those of skill in the art. Alternatively, also using techniques known to those of skill in the art, the support surface can be utilized to control temperature, both heating and cooling, in a manner sufficient to achieve the required denaturation, annealing and extension conditions necessary for the amplification reaction.

A review of FIGS. 5, 6 and 7 will facilitate an understanding of the methods of the present invention, particularly as applied to Bridge Amplification, the technique described in United States Patent No. 5,641,658. Typical PCR cycling profiles can be used in this technique: 10 seconds at 94°C to denature, 10 seconds at temperatures between 50°C and 72°C to allow primer annealing (primer-sequence dependent), and 30-60 seconds at 72°C to allow polymerase extension. A variety of other cycling profiles can be used with the devices and methods of the invention (see, for example, Newton, C.R. and A. Graham, *PCR*, 1994).

FIG. 5A depicts a pair of oligonucleotide primers 7 covalently attached to an amplification site 5. The pair of primers are covalently attached to the surface of the amplification site. In this illustration, the relatively short primers are single strands of DNA. The sequence of each primer is selected to be complementary to the sequence at one end of the target DNA contained in a test sample. Thus, one primer is selected to be complementary to one strand of the target DNA, whereas the other

is selected to be complementary to the opposite strand of the target DNA. The 5' ends of both primers are attached to the amplification site, while the 3' ends remain unattached and are free to participate in the PCR reaction.

FIG. 5A also depicts a single stranded target nucleic acid 8. The target nucleic acid may originate from the test sample or from the PCR denaturation phase. It is in solution and, thus, can contact the primer attached surface. When a target nucleic acid is present, it binds to one of the primers during the PCR annealing phase, as depicted in FIG. 5B. (Although the other complementary strand of denatured target nucleic acid also binds to the other primer, for purposes of simplicity, only the binding of a single strand is illustrated.) During the PCR extension phase, bases from the 3' end of the bound primer are added, to form a surface bound double stranded molecule (9) as depicted in FIG. 5C.

FIGS. 6A-6C illustrate the second cycle of the amplification process. During this PCR denaturation phase, the bound double stranded product 9 shown in FIG. 5C separates into a free single strand 7 in solution and a surface bound single strand 10, as depicted in FIG. 6A. During the PCR annealing phase, the bound single strand contacts the surface, encounters the second primer, and binds to it as depicted in FIG. 6B. In the following PCR extension phase, bases are added from the 3' end of the primer, to form a double stranded nucleic acid molecule matching the target nucleic acid (11), but surface bound at both ends, as depicted in FIG. 6C.

FIGS. 7A-7C illustrate subsequent cycles in the amplification process. Figure 7A depicts doubly bound single stranded nucleic acid 10 following another PCR denaturation phase. During the next annealing phase, each strand binds to an appropriately positioned primer, as depicted in FIG. 7B. During the subsequent extension phase, each strand is converted to double stranded nucleic acid 11 bound to the surface at both ends.

The progression indicated between FIGS. 6C and 7C represents a doubling in quantity of the surface bound amplified nucleic acid. The process can be repeated as many times as desired. With each cycle, the quantity of surface bound amplificate is increased, and additional free single stranded target nucleic acids enter the process, as depicted in FIGS. 5A-5C. With repetitive cycling of this reaction, the quantity of

amplified product increases exponentially, until either the primers become saturated or other reaction components become exhausted. Thus, this process can amplify a single nucleic acid molecule to an analytically useful quantity.

The devices and methods of the invention can be utilized for detecting and identifying multiple genomic targets for Duchenne Muscular Dystrophy as described in Example 1. However, analytical devices and methods of the invention can be utilized in assays of many types of nucleic acids for many purposes such as Human Leukocyte Antigens/Major Histocompatability Complex. Such assays can detect one or more nucleic acids from a plurality of samples. They may also be multiplexed assays conducted with simultaneous, sequential or time overlap detection schemes.

The multielement analytical device of the invention can be produced in many ways. The device may be formed by machining it from bulk material, by assembling it from smaller parts (even those of dissimilar materials), by casting or pressing it in a mold, by solidifying if from a liquid substance, by coating a core material with one or more secondary substances, and by any other method known to those skilled in the art of producing a device containing the required elements. A preferred method of producing the device is by injection molding.

The multielement analytical device of the invention can be formed from a variety of materials including plastics, silica, ceramic, metal, various composite materials, and any other substances known to those in the art to contain properties compatible with the requirements of the device.. A preferred material for the device is a polymer, particularly a plastic.

As described, the essentially flat surface of the device can be subjected to various types of surface treatments. Such treatments can include grinding or lapping to ensure that all surfaces are coplanar.

Optionally, various additional surface finishes can be applied to the essentially flat surface of the element. The surface can be rough or uneven. Such a finish can facilitate the adhesion of surface-bound chemical reagents. The surface can be enclosed by an annular ring or an alternative perimeter boundary. Such an addition can aid in the retention of fluids upon the surface of the element. The surface can contain small wells or pits to contain reagents or facilitate their retention

in the surface. Or, the surface can be formed with a pattern of raised ridges or a pattern of indented channels. Such finishes can segregate particular reagents into particular portions of the essentially flat surface. The surface treatments can also be applied in any combination.

Moreover, the heat conduction properties of the device can be modified, for example, by imbedding one or more high thermal conductivity substances within the material of the device.

The analytical device can be coated with one or more additional substances, for example, to render the amplification surface hydrophilic or hydrophobic, to aid in the adhesion of one or more other substances, to reduce the adhesion of one or more other substances, or to facilitate or prevent one or more chemical reactions. Precise arrays of biological samples may be placed on the amplification sites of the invention using, for example, the GMS Arrayer 417 (Genetic Microsystems, Woburn, MA.

The multielement analytical device of the present invention can be supplied as a kit for commercial or research use. Such a kit can include the device as a single component and, optionally, instructions for its use in the methods of the invention. In a preferred embodiment, the kit also contains a corresponding test sample container component. The components of the kit can be supplied in a sterile or a non-sterile form.

The invention is further illustrated by the following non-limiting example. The contents of all the patents, patent applications and other references cited herein are expressly incorporated by reference in their entireties.

EXEMPLIFICATION

EXAMPLE 1

DETERMINATION OF PRESENCE OR ABSENCE OF MUTATIONS AT THE DUCHENNE MULTIPLE DYSTROPHY LOCUS IN A BLOOD SAMPLE USING A MULTIELEMENT ANALYTICAL DEVICE

In this example, the multiplex PCR method of Chamberlain, *et.al.* (In PCR protocols: A guide to methods and applications, 1990, eds. Innis, *et al.*, Chapter 33, pp. 272-281) is adapted to operate on the multielement analytical device described herein. The method of Chamberlain, *et al.*, involves a 9-way multiplex PCR that amplifies genomic target regions that are most commonly deleted in Duchenne muscular dystrophy patients. Disease-causing deletions of any of the genomic targets should be detected as a failure to amplify that target. Chamberlain, *et. al.*, estimate that 80-90% of all muscular dystrophy patients should be detected by this assay.

Adaptation of the Chamberlain, *et. al.*, assay to the multielement analytical device of the present invention is straightforward. Each post of the multielement device is modified with each of the nine primer pair sets described by Chamberlain, *et. al.* Each primer pair is deposited at a discrete location on the face of the post, separate from each of the other primer sets. After amplification, the posts are stained with a fluorescent DNA-specific stain to detect DNA amplification on the primer spots of the posts. In normal individuals, all nine primer spots should stain intensely. In Duchenne muscular dystrophy patients having common deletions detectable by this set of primers, one or more of the primer sets would fail to amplify and DNA staining would be greatly reduced or absent on one or more of the spots on the post.

MATERIALS AND METHODS

Preparation of Concentrated DNA sample

DNA from the human blood sample to be tested is extracted and purified using the QIAamp DNA Blood Kit (Qiagen, Valencia, CA). The purified DNA sample can be in a concentration between 100.0 and 1000.0 μg of DNA per ml, but is preferably about 500.0 μg/ml. If necessary, the sample can be concentrated to the required level prior to use by precipitation with ethanol or by centrifugal ultrafiltration.

Immediately prior to use, the sample is changed into amplification buffer conditions (1X thermopol buffer, New England Biolabs, Beverly, MA) by addition

of concentrated buffer. The sample is then denatured by incubation at 95°C for 10 minutes, which is followed by rapid cooling at 0°C on ice.

Copolymerization Attachment of Acrylamide-Modified Bridge Amplification Primers to 96-element Multielement Analytical Device

The multielement analytical device contains 96 elements, "posts", that are designed to fit into a corresponding test sample container, a 96-well microtiter plate. The posts fit into the test container such that the flat surface on one end of each of the posts is immersed in the reagent mixtures contained in the test sample container. Each post is modified with multiple primer sets suitable for Bridge Amplification of one or more informative geonomic target loci. A tight seal is formed around each well of the test sample container, to prevent spillage or evaporation of the reagent mixtures, by connecting the multielement analytical device to the test sample container using a connecting component.

Primers are attached to the elements by the copolymerization method of Rehman *et al. (Nuc Acids Res.* 1999, 27: 649-655) The 5'-acrylamide oligonucleotides are copolymerized with monomer acrylamide in contact with an acrylamide-modified surface. Thus, the acrylamide-modified element surface contains a small layer or bead of acrylamide polymerized on the surface. The acrylamide layer contains high concentrations of the 5'-acrylamide on its surface, where it is available for hybridization and amplification reactions.

The amplification primers contain 5' acrylamide modifications. Such 5' acrylamide groups can be added during standard oligonucleotide synthesis using an acrylamide phosphoramidite (Acrydite™ phosphoramidite, Mosaic Technologies, Boston, MA). Alternatively, acrylamide groups can be added after synthesis, as described, by modifying 5' phosphorylated oligonucleotide primers obtained from a commercial source (Operon, Alameda, CA). The terminal acrylate group is added to the appropriate oligonucleotide by forming a mixture of 0.25M N-(3-aminopropyl)methacrylamide-HCl (Polysciences, Warrington, PA.), 0.1M 1-methyl-imidazole (pH 7.0), 0.1M 1-ethyl-3-(3-dimethylaminoproyl) carboiimide-HCl (EDC), and 30 to 50 μM 5'-phosphorylated DNA oligonucleotide. The mixture is

incubated for 1 hour at 50°C in a final concentration of 100 μM. Following incubation, the reaction mixture is diluted 10-fold with a solution containing 0.2M NaOH/ 1mM EDTA. The reaction mixture is placed on a Sephadex G-25 column (NAP 10 disposable columns, Pharmacia), and equilibrated in 0.1M NaOH/1 mM EDTA. The excluded fraction is collected, concentrated and exchanged into tris-EDTA buffer (10mM Tris-HCl, pH 8.3, 1 mM EDTA) by several cycles of centrifugal ultrafiltration and dilution (Microcon 3, Amicon).

The multielement analytical device used in this assay is formed from polystyrene. Covalent acrylamide modification of the element surface is provided using the following procedure. An acid permanganate bath with potassium permanganate dissolved in 1.2 N sulfuric acid (50.0 mg $KMnO_4$ per ml of 1.2 N H_2SO_4) is prepared and heated to 60°C. The element surfaces of the analytical device are immersed in the sulfuric acid bath for about 30 minutes until the surface of the polystyrene elements are oxidized, thereby introducing surface carboxyl groups. The element surfaces are washed in 6N HCl followed by three water washes, to neutralize the sulfuric acid bath and to remove any residual permanagate residue. The carboxyl groups on the element surfaces are derivatized with amino-acrylamide to provide surface acrylamide groups on the polystyrene surface by treating the wells at 50°C for one hour with an aqueous solution containing 0.1M imidazole buffer pH 6.0, 0.1M 1-ethyl-3-(3-dimethylaminopropyl), carbodiimide-HCl (EDC), and 0.1M N-(3-aminopropyl) methacrylamide-HCl (Polysciences, Warrington, PA). The elements are then thoroughly washed with water and air dried.

Solutions containing a pair of 5'-acrylamide primers, monomer acrylamide, and monomer bisacrylamide are prepared and copolymerized on the surface of the acrylamide-modified elements using the following. A separate solution is prepared for each primer set to be attached. A 10% (wt/vol) acrylamide/bis-acrylamide (29:1 weight ratio of acrylamide to bis-acrylamide) is prepared in 10 mM sodium borate buffer with 1mM EDTA, 75% glycerol. 5'-acrylamide primers are added to the acrylamide solution at a final concentration of 100 μm. Immediately prior to deposition on the element surface, the catalysts ammonium persulfate and TEMED

are added, 0.125% (w/v) and 0.125% (w/v), respectively. Primer solutions are spotted onto the element surfaces in the desired configuration. A microarray fabrication device (for example, as obtained from Genetic Microsystem, Woburn, MA) can be used for this placement. After spotting, the device is transferred to a humid saturated nitrogen atmosphere to allow polymerization. This typically occurs within 5-10 minutes at 25°C. After polymerization, any loose or non-crosslinked primer is removed by immersing the device in amplification buffer and thermocycling the device under amplification conditions for 5-15 cycles. After use, the finished device can be washed in distilled water and air-dried for storage, if desired.

ASSAY METHOD

Hybridization and Amplification

The concentrated and denatured DNA samples (500.0 µg/ml) in amplification buffer are distributed in a 96 well microtiter plate. The 96 elements of the multielement analytical device are inserted into the wells of the bottom microtiter plate. The assembled device is incubated overnight at 60°C with shaking (Taitec model M-36 microincubator, Stratagene, San Diego, CA.) to allow hybridization between the oligonucleotide primers and genomic target DNA. After incubation, the multielement analytical device is removed from the microtiter plate and its exterior surface is washed in amplification buffer (1X thermopol buffer, 2 mM Mg SO_4, 50 µg/ml bovine serum albumin) to remove any unbound DNA.

The multielement analytical device is transferred to a second microtiter plate containing approximately 100µl per well of amplification reagent mix (30 µl PCR buffer, 24 µl water, 3 µl NEB thermopol buffer II, 2.4µl 2.5 mM dNTP 0.6 µl 100 mM $MgSO_4$, 0.2 µl 10 mg/ml BSA, and 0.2 µl vent polymerase). The assembly is incubated for 5 minutes at 60°C to allow initial extension of the hybridized genomic DNA. Then the assembly is thermocycled 35-45 times using a cycle of 30 seconds each at 60°C, 72°C, 94°C.

Detection

The 96 post device is washed several times by immersing the tips in TE buffer (10 mM Tris-HCI at pH 8.0, 1 mM EDTA). Bridge amplification products are stained in 1 X SYBR Green I (Molecular Probes, Eugene, OR) in TE buffer for 10 minutes at room temperature. The posts are then washed in TE buffer without SYBR at 70°C for 15 minutes, and then transferred to fresh TE buffer. The post device is then imaged using a bottom reading fluorescent imager, such as an inverted fluorescence microscope or a Fluorimager 595 (Molecular Dynamics, Sunnyvale, CA) to detect where amplification occurred.

EQUIVALENTS

While this invention has been particularly shown and described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed by the scope of the claims.

CLAIMS

What is claimed is:

1. An analytical device comprising a plurality of elements, each element containing at least one essentially flat surface comprising an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, the elements configured such that the at least one essentially flat surface of each element can be simultaneously immersed in a single test sample container.

1. The device of claim 1, wherein the elements are cylindrical.

2. The device of claim 2, wherein the elements are hollow.

3. The device of claim 2, wherein the elements are solid.

4. The device of claim 2, wherein there are 96 elements.

5. The device of claim 5, wherein the test sample container is a 96-well titer plate.

6. The device of claim 5, wherein there are 24 amplification sites in the array.

7. The device of claim 1, wherein the device is formed from a polymer.

8. The device of claim 1, further comprising a connecting component.

9. The device of claim 1, wherein the at least one flat surface has a surface design selected from the group consisting of a rough surface, a pitted surface, a smooth surface surrounded by an annular ring, a smooth surface with

discrete sections formed by raised ridges, a smooth surface with discrete sections formed by indented channels and any combination thereof.

10. The device of claim 1, further comprising an acrylamide gel coating at the amplification sites.

11. The device of claim 11, wherein the surface of the amplification site is positively charged.

12. An assay method for detecting the presence of a target nucleic acid sequence in a plurality of test samples comprising the following steps:

a. positioning an analytical device comprising a plurality of elements, each element containing at least one essentially flat surface comprising an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, the elements configured such that the at least one flat surface of each element can be simultaneously immersed in a single test sample container, into a single test sample container such that the amplification sites are in contact with the test samples under conditions suitable for hybridization to occur;

b. maintaining contact between the amplification sites and the test samples for sufficient time for hybridization to occur;

c. amplifying the hybridized target nucleic acid sequence; and

d. detecting the presence of the amplified target nucleic acid sequences.

13. The method of claim 13, wherein the assay is performed in a multiplex manner.

14. The method of claim 14, wherein multiple target nucleic acids are detected.

15. The method of claim 13, wherein the analytical device is separated from the test container prior to the amplification step.

16. The method of claim 13, wherein the amplified target nucleic acid sequences are optically detected.

17. The method of claim 17, wherein the amplified target nucleic acid sequences are detected by fluorescence.

18. The method of claim 13, wherein the target nucleic acid sequence is amplified to 10^4 to 10^6 fold.

19. A method of making an analytical device comprising a plurality of elements, each element containing at least one essentially flat surface comprising an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured such that the essentially flat surface of each element can be simultaneously immersed in a single test sample container selected from the group consisting of molding, machining, assembly, and combinations thereof.

20. The method of claim 20, wherein the analytical device is made by injection molding.

21. A kit comprising an analytical device comprising a plurality of elements, each element containing at least one essentially flat surface comprising an array of amplification sites containing a plurality of pairs of surface bound nucleic acid probes, configured such that the at least one flat surface of each element can be simultaneously immersed in a single test sample container.

22. The kit of claim 22 further comprising a test sample container.

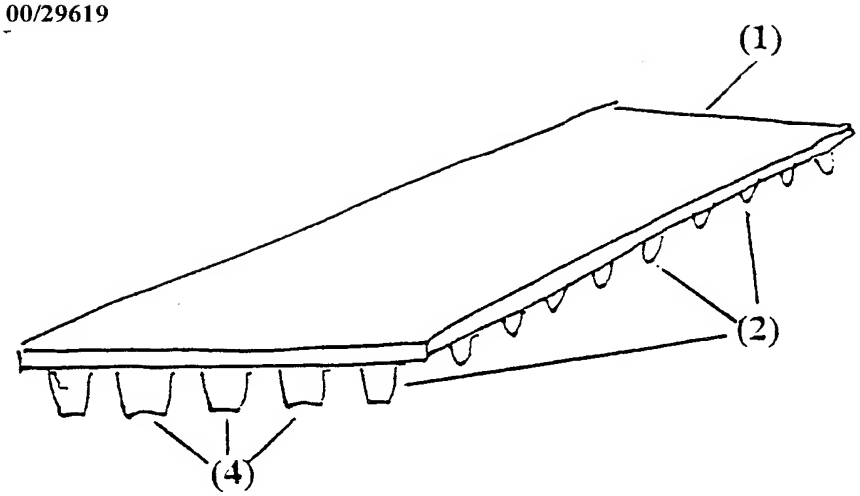


FIG. 1A

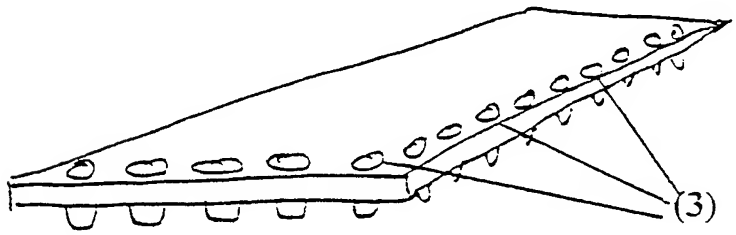


FIG. 1B

FIG. 1C

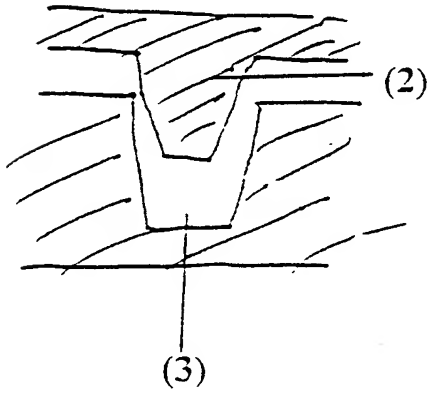
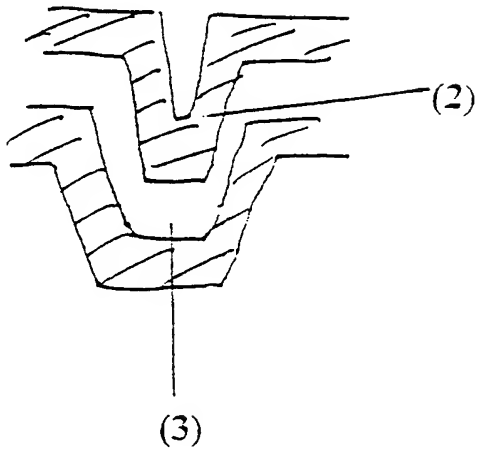


FIG. 1D



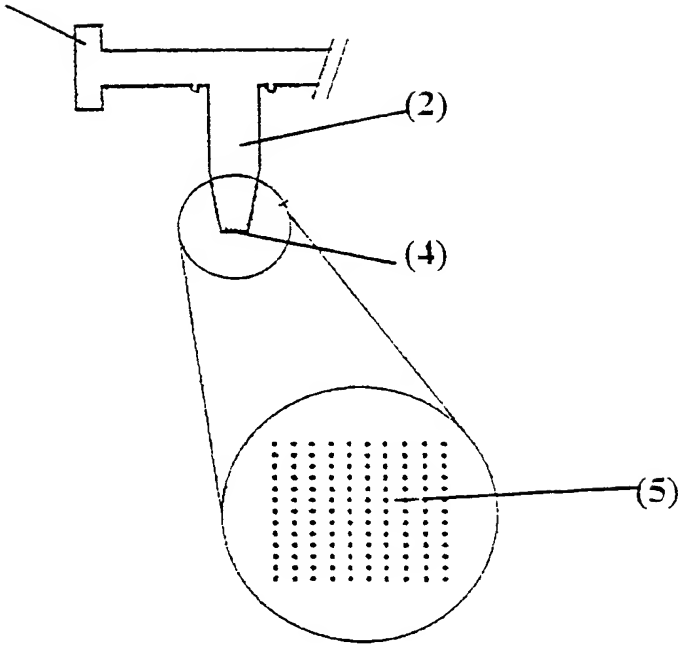


FIG. 2

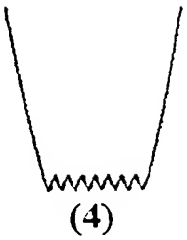


FIG. 3A

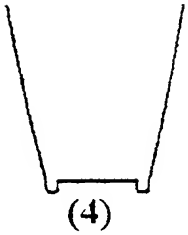


FIG. 3B

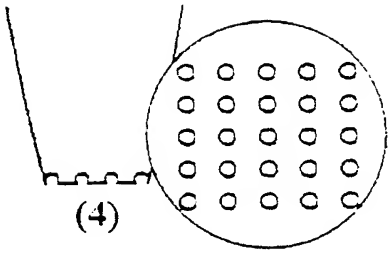


FIG. 3C

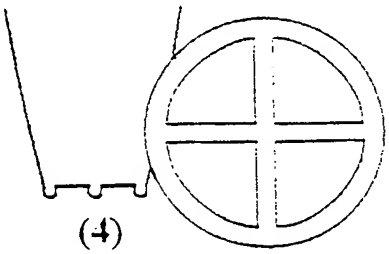


FIG. 3D

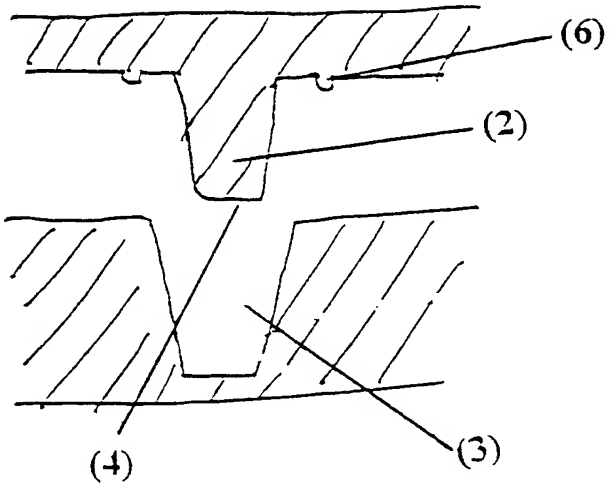


FIG. 4A

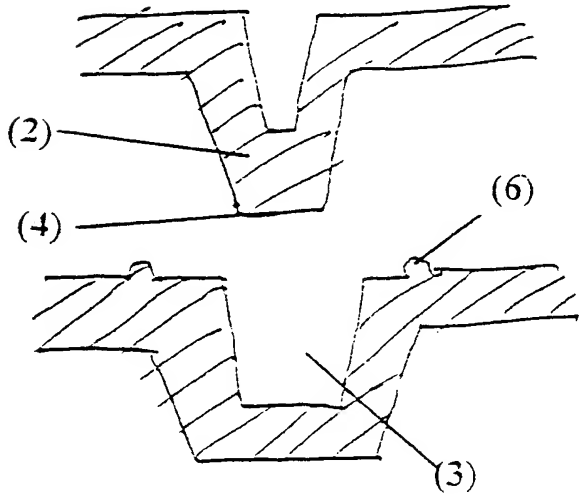


FIG. 4B

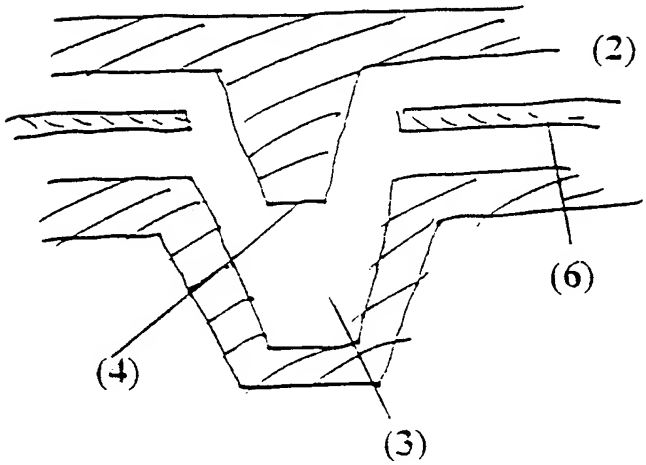


FIG. 4C

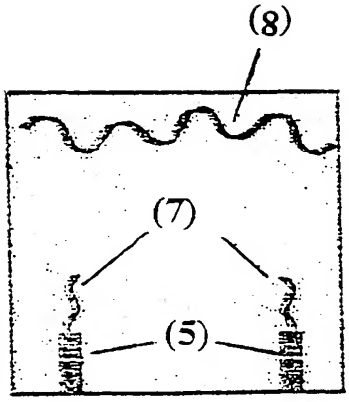


FIG. 5A

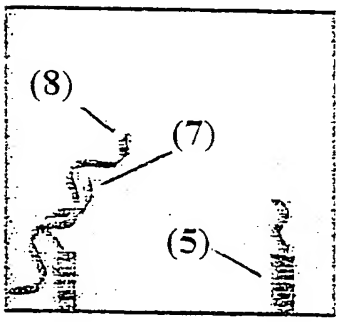


FIG. 5B

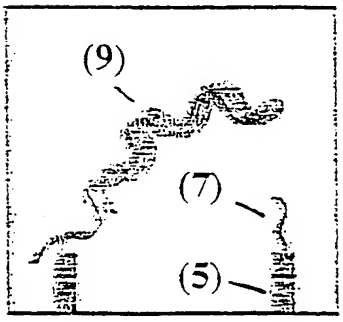


FIG. 5C

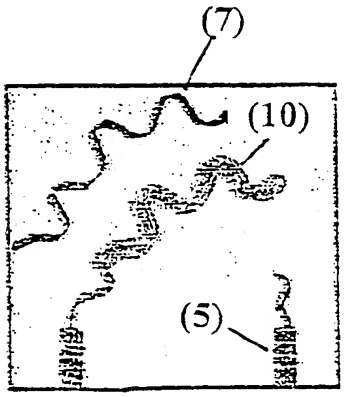


FIG. 6A

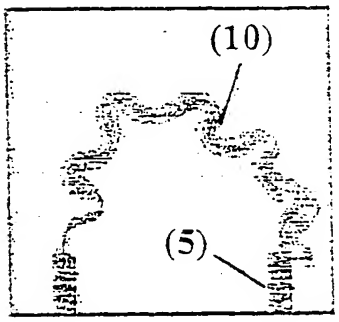


FIG. 6B

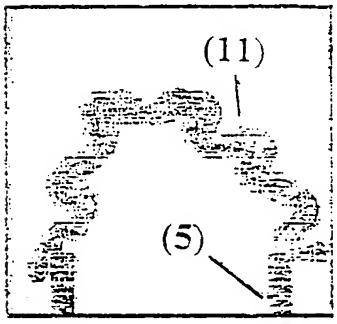


FIG. 6C

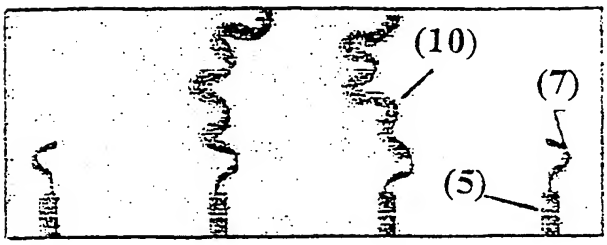


FIG. 7A

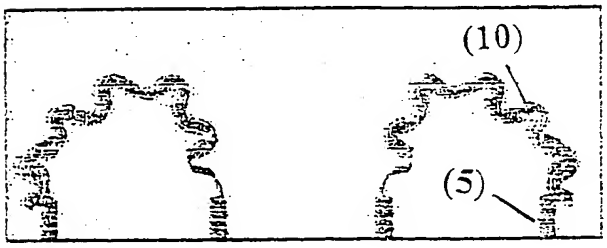


FIG. 7B

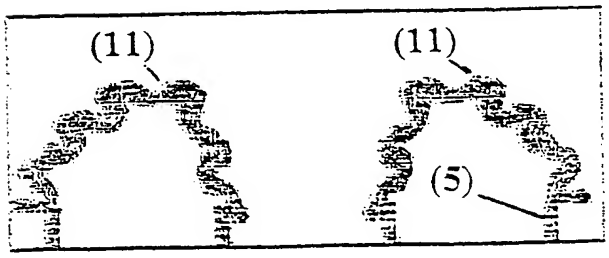


FIG. 7C